of opening; the sensitivity of each subset to specific cardioactive drugs may be somewhat different and permit a semi-selective block of one or the other subset of Na channels. Because we have employed membrane taken from embryonic myocardium, questions involving the maturation and development of the Na channel are cogent.

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CONDUCTION, BLOCKADE AND GATING IN A Ca^{2+}-ACTIVATED K^+ CHANNEL INCORPORATED INTO PLANAR LIPID BILAYERS

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Ca^{2+}-activated K^+ channels of large unitary conductance have been identified in several different types of cells (Schwartz and Passow, 1983; Latorre and Miller, 1983). To study this channel in a simplified, cell-free system and as a first step towards reconstitution, we have incorporated it into planar lipid bilayer membranes (Latorre et al., 1982; Vergara and Latorre, 1983; Moczydlowski and Latorre, 1983). We found that the Ca^{2+}-activated K^+ channel incorporated into artificial membranes has about the same conductance and gating kinetic characteristics as those found in cultured rat muscle by means of the patch-clamp technique (e.g., Barret et al., 1982; Methfessel and Boheim, 1982). Here, we report some of the conduction, blockade, and gating properties of this channel.

CONDUCTANCE-ACTIVITY RELATIONSHIP

Fig. 1 shows g vs. a_K data for bilayers of pure phosphatidylethanolamine (PE) in the absence of Tris.[1] The *inset* of Fig. 1 is a Scatchard or Eadie-Hofstee plot of the same data. It is apparent that the conductance-activity relationship deviates markedly from a Langmuir isotherm. The latter would be expected for a single-ion channel with fixed energy barriers (Lauger, 1973). In the *inset*, we have drawn two straight lines to show limiting behavior in the low and high activity range that we define for the sake of discussion, as high- and low-affinity "sites." The high-affinity site is roughly characterized by a maximal conductance of 220 pS and an apparent dissociation constant of 3 mM; the respective parameters for the low-affinity site are

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[1]The presence of mM amounts of Tris appears to depress the unit conductance of the channel at low K^+, resulting in nearly perfect Michaelis-Menten behavior as shown previously (Latorre and Miller, 1983).

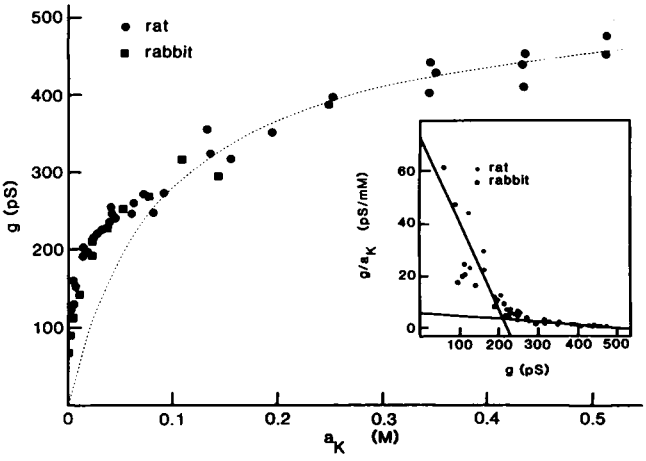

FIGURE 1 Channel conductance-activity relationship. Single channels were incorporated into a planar bilayer (200 μm diam) formed on a polystyrene partition by spreading a solution of 20 mg/ml bovine brain phosphatidylethanolamine (Avanti Polar Lipids, Birmingham, AL) in decane over the hole. The electrical recording system, the rat muscle membrane preparation, and incorporation method have been previously described (Vergara et al., 1982). Conductances were measured with KCl-MOPS buffers at various salt concentrations adjusted to pH 7 with KOH. To promote channel incorporation when [KCl] was ≤10 mM, an osmotic gradient was created. At the beginning of the experiment, the *cis* compartment (where the membrane vesicles were added) contained 100 mM CKl, whereas the *trans* compartment was kept at a low ≤10 mM [KCl]. After channel incorporation, the *cis* compartment was perfused with a KCl buffer of the desired concentration. Channel conductance was calculated from the slope of the current-voltage relationships, which were linear in the voltage range −40 to +40 mV and at all the a_K studied. Both rat (Moczydlowski and Latorre, 1983) and rabbit (Latorre et al., 1982) membrane preparations were used in these experiments. The dotted line shows a fit to the low affinity region according to: $G/G_{max} = a_K/(a_K + K_d)$ where G_{max} = 530 pS and K_d = 90 mM.

530 pS and 90 mM. The physical basis for such behavior is by no means clear at present, although several possible explanations may be given. Many types of energy profiles for the conduction pathway could result in apparent negative cooperativity; for example, two initially different noninteracting sites or two initially identical interacting sites. This explanation invokes multiple-ion occupancy of the channel; however, a single site that can relax slowly to two or more different conformations is also possible (Lauger, 1980). Another possible explanation for deviation from a rectangular hyperbola in the low concentration limit would be fixed negative charges in lipid or protein near the ion-binding site. Monolayer experiments show that the PE we use is 2%–3% negatively charged at pH 7.0. However, this amount of randomly distributed charge in the bilayer cannot account for the observed deviation. We think the explanation of fixed charge on the protein unlikely, because we would expect to observe a limiting conductance value in the low K^+-activity limit. The available data of Fig. 1 in this region show no clear evidence of such a limit.

TEA AND Ba^{2+} BLOCKAGE

Insight on the gross architecture of the Ca^{2+}-activated channel can be obtained by studying the effects of K^+-channel blockers such as tetraethylammonium (TEA) and Ba^{2+} in the same manner as for the squid axon delayed rectifier (e.g., Armstrong, 1975). Fig. 2 shows that TEA ions decrease channel conductance when added to either the *cis* or the *trans* side of the membrane (Fig. 2 *A* and *B*).

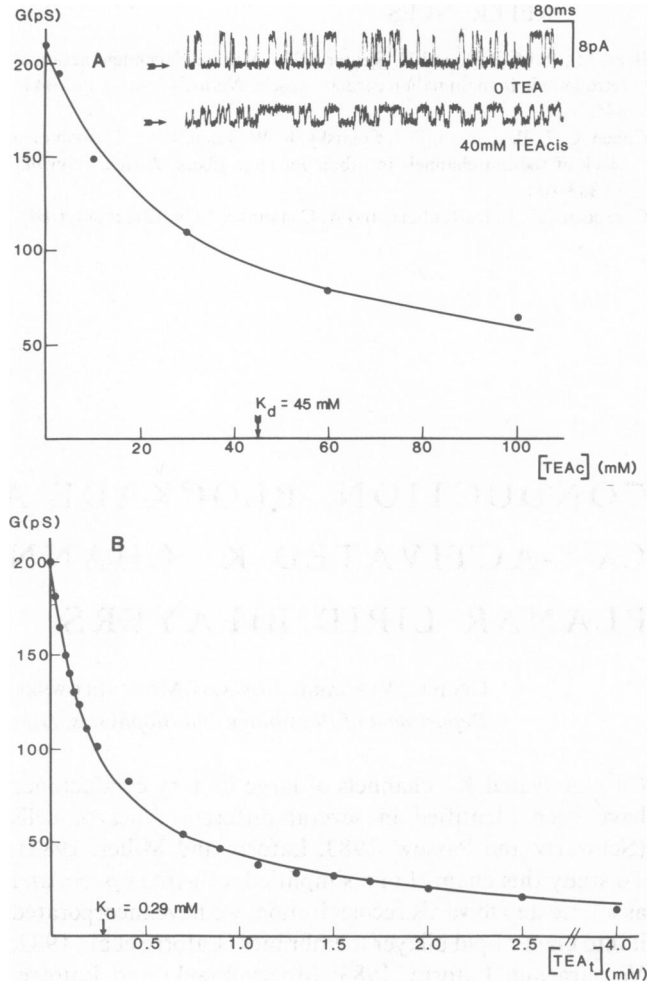

FIGURE 2 Effect of TEA on channel conductance. *A*, channel conductance was measured as described in Fig. 1 in symmetrical 0.1 M KCl. TEA was added to the *cis* side only at the concentrations indicated. Applied voltage was +40 mV. Solid line was drawn according to $G = G_o (1 + [TEA]/K_d)^{-1}$, where G and G_o are the channel conductances measured in the presence and absence of TEA. *Cis*-TEA block is voltage dependent with a dissociation constant described by $K_d(V) = K(0) \exp(-z\delta V/kT)$, where $K(0)$ is the dissociation constant at zero volts, z is the valence of the blocking ion, δ is the fractional electrical distance at which the blocking site is located. V is the voltage, and k and T have their usual meaning. The solid line was drawn according to the first equation with a $K(0)$ = 35 mM and δ = 0.34. *B*, all conditions as in *A* but TEA was added to the *trans* side only. The solid line (—) was drawn according to the single-site titration equation given above with a K_d = 0.29 mM (for more details see text).

However, the TEA effect is dramatically different for *cis* vs. *trans* TEA addition. The decrease in single-channel conductance implies that the blocking reaction between TEA and the channel is faster than the gating kinetics. *Cis* and *trans* channel blockade by TEA follows a single-site titration curve (—, see Fig. 2).

In addition, we found that the *cis* TEA blockade is voltage dependent. The apparent dissociation constant, K_d, decreases as a function of voltage. From the voltage dependence of K_d, we conclude that 34% of the total voltage drop across the membrane is found at the TEA binding site. In contrast, *trans* TEA blockade is voltage independent. K_d's for the blocking reaction at zero voltage are 0.29 mM for *trans* and 35 mM for *cis* TEA blockade. These results suggest that the *cis* (internal) and *trans* TEA receptors are different and that the channel might at each site contain mouths wide enough to accommodate TEA (~0.8 nm).

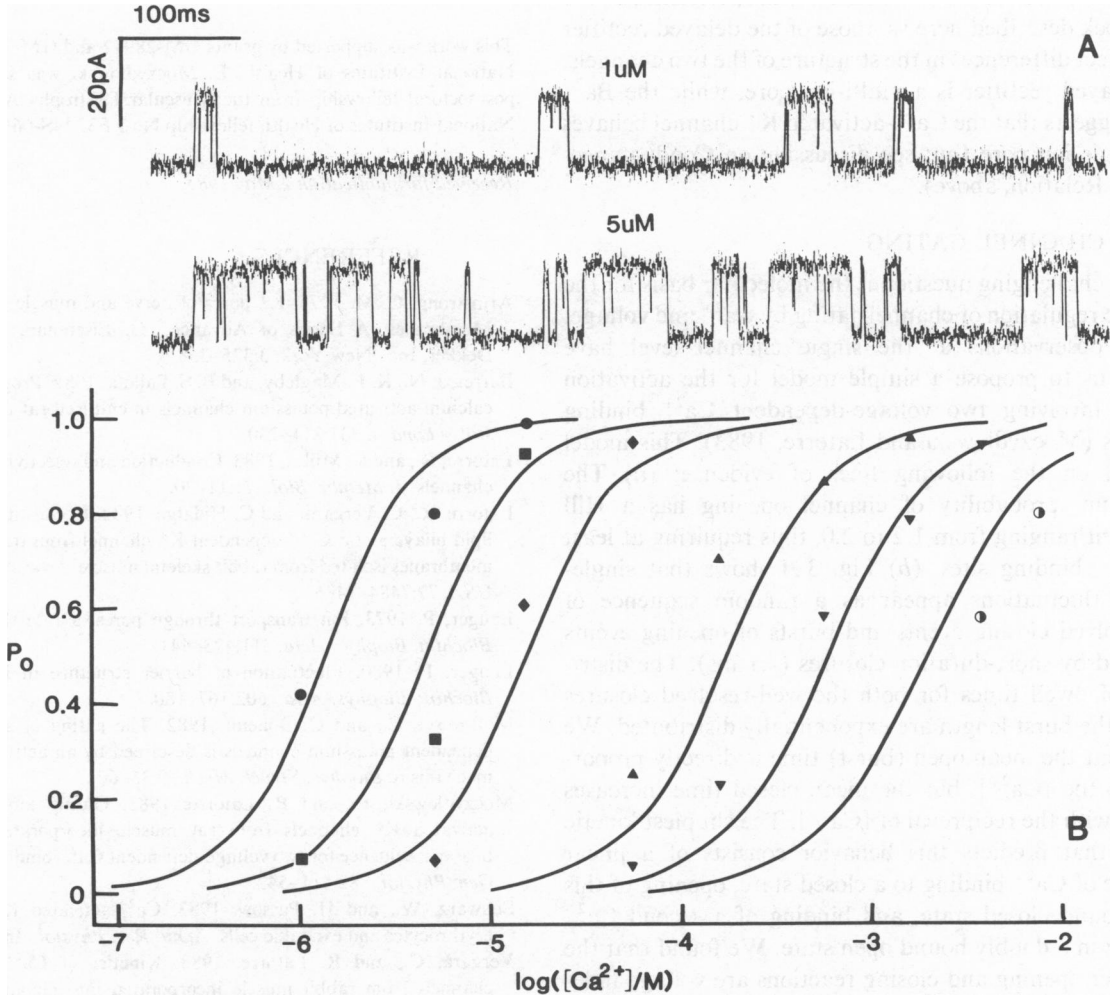

FIGURE 3 Effect of Ca^{2+} and voltage on the open-state probability of a K^+ channel from rat muscle plasma membrane. The solution on both sides of the bilayer was 10 mM Tris-MOPS, pH 7.0, 0.2 M KCl, and various [Ca^{2+}]. *A*, current fluctuations of a single channel at two different *cis* Ca^{2+} concentrations. Low pass filter = 2 kHz. *Top trace*, 1 μM $CaCl_2$; *bottom trace*, 5 μM $CaCl_2$. Applied voltage = +50 mV. Arrows to the right of each trace show the zero current level. *B*, comparison of the actual and theoretical open-state probability, P_o, as a function of [Ca^{2+}] and voltage. The actual open-state probability was measured by computer from digitized data (1,000 points/s) as the total time in the open state divided by the total recording time (20 s). The theoretical open-state probability (—) was derived for the following kinetic model as previously described (Moczydlowski and Latorre, 1983):

$$\text{Closed} \overset{K_1(V)}{\rightleftharpoons} \text{Ca. Closed} \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \text{Ca.Open} \overset{K_2(V)}{\rightleftharpoons} \text{Ca}_2\text{.Open}$$

The equations and best-fit constants used to construct the theoretical curves for this model are $P_o([Ca],V) = [C^2 + CK_2]/[C^2 + CK_2(1 + \alpha/\beta) + K_1K_2(\alpha/\beta)]$ where $C = [Ca^{2+}]$; $\alpha = 280\ s^{-1}$; $\beta = 480\ s^{-1}$; $K_1 = 3.7 \times 10^{-4} \exp(-0.052\ V)$; $K_2 = 1.4 \times 10^{-5} \exp(-0.071\ V)$. Applied voltages for each curve were as follows, from left to right (mV): +60(●), +40(■), +20(♦), −20(▲), −40(▼), −60(○).

We have also characterized in detail the channel blockade induced by Ba^{2+} ions (Vergara and Latorre, 1983). Although Ba^{2+} blockade of the Ca^{2+}-activated K^+ channel shows several similarities to the Ba^{2+} blockade of both the delayed and inward rectifiers, there are several important differences. First, while Ba^{2+} blockade is apparent from both the *cis* and *trans* sides of the channel, our data can be explained in terms of a single binding site for Ba^{2+}. Second, there is no evidence in this channel of current-dependent phenomena or "knock on" or "knock off" behavior in the Ba^{2+} block. The characteristics of the Ba^{2+} block described here vs. those of the delayed rectifier may reflect differences in the structure of the two channels. The delayed rectifier is a multi-ion pore, while the Ba^{2+} block suggests that the Ca^{2+}-activated K^+ channel behaves as a single-ion pore (but see discussion on Conductance-Activity Relation, above).

CHANNEL GATING

Another challenging question is the molecular basis for the exquisite regulation of channel gating by Ca^{2+} and voltage. Several observations at the single channel level have allowed us to propose a simple model for the activation kinetics involving two voltage-dependent Ca^{2+} binding reactions (Moczydlowski and Latorre, 1983). This model is based on the following lines of evidence: (*a*) The equilibrium probability of channel opening has a Hill coefficient ranging from 1.2 to 2.0, thus requiring at least two Ca^{2+} binding sites. (*b*) Fig. 3 *A* shows that single-channel fluctuations appear as a random sequence of well-resolved closing events and bursts of opening events separated by short-duration closures (<1 ms). The distribution of dwell times for both the well-resolved closures and for the burst length are exponentially distributed. We found that the mean open (burst) time is directly proportional to the $[Ca^{2+}]$, but the mean closed time increases linearly with the reciprocal of $[Ca^{2+}]$. The simplest kinetic scheme that predicts this behavior consists of a linear sequence of Ca^{2+} binding to a closed state, opening of this singly-bound closed state, and binding of a second Ca^{2+} resulting in a doubly bound open state. We found that the first-order opening and closing reactions are voltage independent, while the two Ca^{2+} binding equilibrium constants are exponential functions of the applied membrane potential. These results lead us to believe that the actual opening and closing of the K^+-selective pore is a simple voltage-independent conformational change. We propose that the voltage dependence of the channel is conferred by Ca^{2+} binding to two distinct sites located within the electric field. Fig. 3 *B* shows that a quantitative treatment of this model predicts the equilibrium open-state probability, P_o, as a function of Ca^{2+} concentration and voltage, with an accuracy of ~0.1 P_o units. (For more details, see Fig. 3 *B*)

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